Surgical options for patients with ectasic disorders continue to evolve. Collagen cross-linking and INTACS hold promise for delaying or avoiding transplantation. Laser applications to previously manual procedures bring new precision to tissue modifications and visual rehabilitation. Here, we discuss new technologies that may strengthen weak corneas to address ectasia and advanced vision correction procedures to aid those with keratoconus and keratoectasia.

**EXCIMER LASER LAMELLAR KERATOPLASTY**

The use of the excimer laser to sculpt the cornea has been the most important advancement in the field of refractive surgery. With reports of epithelial sensitivity to ultraviolet lasers and precise etching properties using various substrates, Trokel in 1983 studied the effect of the excimer laser on bovine corneas. They demonstrated that far ultraviolet laser emissions (between 150 and 200 nm) could precisely remove corneal stroma without apparent thermal trauma to the adjacent tissue. The laser did not create adverse alterations of wound healing processes including cellular migration, proliferation, or production of new tissue. This landmark report started a multitude of investigations into the use of laser for refractive surgery. The 193-nm ultraviolet light from the argon fluoride laser demonstrated the critical characteristic of having the least transmission through corneal tissue. This resulted in the least amount of adjacent tissue damage and created a smoother ablation compared to longer wavelength lasers. Argon fluoride lasers (also called excimer lasers as an acronym for excited dimer) were subsequently developed for keratorefractive procedures. At a 193 nm wavelength, high-energy photons break organic molecular bonds of the superficial corneal tissue in a process called ablative photo-decomposition. Particles are expelled at high velocity, which helps to dissipate much of the energy. Other important properties of the laser were also explored, including the optimum irradiance levels and repetition rates. The optical principles for the laser correction of ametropia have been developed and are straightforward. At the same time, therapeutic uses of the excimer laser have also been developed. In the phototherapeutic keratectomy (PTK) procedure, an excimer laser is used as an adjunctive tool to surgically treat a variety of superficial corneal disorders. With PTK, a uniform depth ablation of the surface corneal tissue is executed over a wide area that is typically from 8 to 11 mm. This technique produces a smooth surface and clear visual axis and minimizes tissue removal. Due to these specific characteristics of the PTK procedure, some authors have proposed the use of the PTK mode for lamellar keratectomy instead of the microkeratome. The overriding advantage of using the excimer laser for lamellar keratectomy is its ability to remove tissue with microscopic precision that is unattainable with other procedures. The depth of ablation is then adjusted as necessary by the surgeon so that a very thin, keratoconic cornea with extreme curvatures can undergo a laser lamellar keratectomy, mitigating risks that were asso-
associated with historical techniques. These properties of the excimer laser are ideal for the surgical treatment of corneal ectasia, such as keratoconus.

The laser lamellar keratectomy for keratoconus began in the 1990s based upon previous experiences with epikeratoplasty. In this technique, donor corneal tissue is pre-lathed into the proper shape and sutured onto a recipient, de-epithelialized cornea in which Bowman’s layer and the stroma have been left intact. The change in curvature of the anterior surface from the lenticule provides the refractive correction. Unfortunately, despite various changes in this technique, the refractive results were disappointing. Many authors have demonstrated the utility and accuracy of the excimer laser for reproducible corneal photoablation in lamellar keratoplasty.6,7 To improve the anatomical and functional outcome of lamellar keratoplasty for patients with early stage keratoconus, the excimer laser lamellar keratoplasty (ELLK) with augmented thickness technique was developed.7 ELLK is a procedure in which a deep plano excimer laser ablation is performed on the host cornea and a donor button is sutured into the recipient bed. No thermal or mechanical damage to the cornea in the areas adjacent to the ablation was observed on light microscopy.13 Additionally, the laser did not create adverse alteration of wound healing responses including cellular migration, proliferation, and production of new tissue.13 Excimer laser-assisted keratoplasty for keratoconus provides an adequate improvement of corneal thickness, which is useful to restore the corneal optic integrity and structure.

Laser lamellar keratectomy seemed to be a simple and safe technique: in PTK-mode with an ablation area of 10-mm diameter, the treatment was centered on the pupil and executed using a non-ablative ring mask to protect the peripheral cornea. The ablation depth was planned to leave a 200-µm thickness at the thinnest point, avoiding any consequences with the endothelium. A peripheral pocket was then created and the lamella positioned on the recipient bed to restore a regular anterior corneal surface. Functional and anatomic improvements were evident, but in some cases the mechanical effects of the recipient’s original keratoconus persisted, especially with advanced and decentered ectasia. These disadvantages limited the use of this technique to only mild or moderate keratoconus cases. Other disadvantages of excimer laser lamellar keratoplasty were vision-limiting interface problems. To further reduce the mechanical effects and improve these cases, new methods were necessary to regularize the ectasia and to create unique ablations, customizing the lamella and recipient bed.

The introduction of a new-generation excimer laser with a comprehensive surgical planning application specific for laser lamellar transplantations allowed the surgeon to create customized ablations for both the receiving bed and the lamella as complements. Specifically, this provided the capability to plan different ablation depths in the same cornea as a function of corneal thickness differentials (pachymetric-link) or with corneal aberrations (topoaberrometric-link). The concept of customized ablation was developed in the mid 90s,14 and it seemed useful to apply the made-to-measure, custom ablation strategy to lamellar keratoplasty to improve the surgical technique and the results. Corneal Lamellar Ablation for Transplantation (CLAT; iVIS Technologies, Taranto, Italy) was developed for therapeutic surgery in order to restore the normal corneal pachymetric gradient utilizing devices that provide highly repeatable anterior elevation, posterior elevation, and pachymetry data. For this technique, a custom refractive surgery platform is used that is an integrated system of devices and software to customize refractive and therapeutic surgery specific to each patient’s unique and individual needs. The iVIS Suite (iVIS Technologies) is an integrated platform that consists of software applications for CLAT; Precisio (iVIS Technologies) high-resolution surgical tomographer; pMetrics (iVIS Technologies) dynamic pupillometer; and a high-resolution ablation delivery platform using the iRES 1.000-Hz laser (Figure 18-1). A fundamental premise in the design concept of these diagnostic, design, and delivery systems is to provide highly repeatable, high-definition data appropriate for the demands of high-resolution custom refractive and custom therapeutic corneal surgery. CLAT is a paradigm shift allowing the surgeon to execute a totally automated, customized lamellar transplantation of the cornea.

CLAT is a 3-step surgical process (Figure 18-2) to create both a thin, uniform thickness receiving bed and a dimensionally matched donor lamella. When the shaped donor lamella is placed within the receiving bed, a full-thickness regularized cornea results, which importantly preserves the patient’s endothelium, Descemet’s membrane, and the posterior stroma. Unlike microkeratome or laser-keratome lamellar surgeries, CLAT removes the irregularities in the cornea in preparation for receiving the matched uniform
thickness donor. This process yields significantly improved refractive characteristics of the resultant postoperative cornea. Keratome or laser-keratome based surgeries which reference the anterior surface, leave or mirror all of the preoperative corneal irregularities in the postop cornea and therefore develop a significantly more aberrated refractive condition.

The donor cornea is positioned on a specialized concave support with the endothelial side exposed. The surgeon then uniformly reduces the donor cornea thickness with the excimer laser. The donor cornea is then positioned on a convex support with the epithelial side exposed for excimer laser trephination, using a donor mask of equal (or 0.25-mm larger) diameter to the recipient’s ablation. A perimetral saddle with depth and width defined by the surgeon is performed by positioning a secondary mask with a smaller diameter. The receiving bed is created by utilizing a three-dimensional (3-D) pachymetry map and calculating the intersection of an ideal, uniform thickness corneal bed for the patient uniquely referencing the posterior surface of the cornea. The irregular volume above this ideal surface is removed with the iRES laser (gaussian flying-spot 650 µm, 1000 Hz, 193 nm; iVIS Technologies, Taranto, Italy) with the patient under topical anesthesia (ropivacaine 1%). A round, non-ablatable plastic mask (from 7.5 mm to 8 mm in diameter) is placed on the cornea to create a vertical edge to the ablation and then the ablation is performed transepithelially. The choice of the mask’s diameter is secondary to the Ideal Pupil dimension (iVIS Technologies) that is calculated from the patient’s pMetrics pupillometry study. The minimal estimated residual stromal thickness of the completed receiving bed is usually specified to be 200 µm. At this uniformly thin dimension, the remaining tissue takes on membrane properties with no cross-sectional rigidity. When the ablation is complete, the new membrane characteristics of the bed will cause the bed to be positioned along the isostatic line that eliminates the deformation induced from the corneal pathology.

At this moment, local anesthesia is achieved with a peribulbar injection of 10 cm³ of bupivacaine 0.5% and mepivacaine 4% combination. Each patient is prepared and draped in the usual fashion. Several drops of a 5% povidone-iodine solution are instilled in the inferior fornix, and a lid speculum is inserted to keep the eye wide open.

With a circular movement using a disk knife a 2.5-mm stromal pocket is obtained around the 360-degree circumference of the ablation floor.

The donor lamella is then secured into the recipient bed with 4 10-0 nylon cardinal sutures at the 6, 12, 9, and 3 o’clock positions, and then, after the introduction of the wing of the donor lamella into the stromal pocket, 16 interrupted 10-0 nylon sutures are placed. Finally, the knots are buried, and intraoperative suture adjustment is performed. At the end of surgery, the speculum is removed, and the eye is patched. The patch is removed the day after the surgery. The postoperative therapy consists of topical ofloxacin 3% 3 times daily until re-epithelialization is completed. Topical dexamethasone 0.1% is administered for at least 1 month, and then tapered and titrated. Within 3 months from surgery, all patients stop their medication. Preservative-free artificial tears (sodium hyaluronate 0.2%) are used for up to 6 months in each case. An image of a typical patient at 1 month postoperatively is shown in Figure 18-3.

Two months after surgery, the sutures responsible for major graft distortion are starting to be removed, as indicated by corneal topography analysis (example of the change in topography is shown in Figure 18-4). Over the following 3 months, all the remaining sutures are selectively removed to achieve as regular corneal curvature as possible. A biomicroscopy image of a patient at 3 months
post-ELLK is shown in Figure 18-5. In all patients, suture removal has been completed by the 6-month follow-up examination.

Subsequent to 6 years of experience using standard ELLK on 80 keratoconic eyes, 30 eyes of 30 patients (mean age 34.9 ± 8.4 years; range 21 to 55) affected by mild to moderate keratoconus underwent CLAT procedures. The follow-up examinations were performed at 1, 3, 6, 9, 12, and 24 months postoperatively. The mean preoperative uncorrected visual acuity (UCVA) was 0.10 ± 0.11 SD, and mean preoperative best spectacle-corrected visual acuity (BSCVA) was 0.46 ± 0.20 SD, with a mean manifest refraction spherical equivalent (MRSE) of −4.73 D ± 4.25 SD. The mean preoperative corneal thickness was 414 µm ± 24 SD (range from 382 to 484); the mean preoperative keratometric astigmatism was 5.78 D ± 3.11 SD (range from 1.65 to 7.47 D) with a mean keratoconus apex power of 67.64 D ± 9.96 SD. The mean preoperative endothelial cell density was 1954 cell/mm² ± 214 SD (range from 1919 to 2489), the mean photopic pupil diameter was 3.66 mm ± 0.45 SD; the mean scotopic pupil diameter was 6.34 mm ± 0.57 SD; the mean Ideal Pupil diameter was 5.44 mm ± 0.53 SD.

The mean donor ablation depth was 271 µm ± 59.4 SD (range from 100 to 420); the mean recipient ablation depth was 198 µm ± 30.4 SD (range from 138 to 260). Surgeries were performed by one surgeon (LS) always specifying a minimum estimated residual corneal bed of 200 µm. Statistical analysis was performed using the paired Student’s t-test. The results obtained are shown in Figures 18-6, 18-7, and 18-8.
The depth of laser ablation is set by the surgeon in relation to the preoperative corneal thickness of the eye and laser lamellar keratectomy can be performed with minimized risk. In our procedures, preoperative planning of the laser ablation with a safety margin of 200 µm of residual corneal bed was always considered. No endothelial damage has developed during the 2 years of follow-up. A normal thickness (>500 µm) was restored in all patients by implanting a donor lamella thicker than the tissue ablated from the recipient cornea. The improvement in the corneal curvature (<50 D) was obtained by the flattening effect of the sutures placed as described for other lamellar techniques, such as epikeratophakia.\textsuperscript{15}

In our series, the patients have shown a slow but steady improvement in their visual acuity after surgery. The final visual results were satisfying and similar to those achieved with other lamellar keratoplasty techniques, such as microkeratome-assisted LK\textsuperscript{16} and PK.\textsuperscript{17-19} Buratto et al\textsuperscript{6} reported a mean BCVA of 20/30 in 20 keratoconus patients after ELLK. Recently, Bilgihan et al\textsuperscript{20} obtained similar results after treating 5 keratoconus patients with ELLK. These results could have been affected by the use of old-generation lasers and the photoablation of the stromal side of the donor grafts.

The slowness of visual recovery could be due to various factors, such as 1) haze of graft-bed interface, 2) lower ability of the host keratocytes to colonize the rehydrated lamella, and 3) small folds in the recipient bed caused by mechanical tensions of the suture on the lamella. We suppose that, in our patients, these factors have improved spontaneously and steadily over time. The early removal of sutures, and in all cases within the fifth month from surgery, may have affected the patients’ refraction because of changes in both the spherical equivalent and the refractive cylinder observed during the follow-ups.

Keratoconus patients who need surgery are often young and CLAT does not rule out the possibility of a PK in the future. CLAT, as other types of anterior LK, has additional advantages over penetrating keratoplasty in the eye banking procedures: a quality donor endothelium is not required, and this reduces the problem of tissue availability. Donor corneas discarded for endothelium problems and not suitable for PK can instead be used for lamellar procedures. Moreover, the corneal lamella can be dehydrated in a silicone gel and stored for longer times.

In conclusion, our 2-year experience indicates that the optical pachymetry-guided customized corneal lamellar transplantation using an ultrastiff excimer laser can be a technique to consider when treating moderate to advanced keratoconus with a minimal corneal thickness of 350 µm. In these cases, CLAT can be a valuable alternative to penetrating keratoplasty, with similar efficacy and less invasiveness. Importantly, the preservation of the recipient’s healthy endothelium creates a higher safety profile. The use of excimer laser has allowed us to standardize lamellar
keratoplasty by simplifying the surgical process, shortening the surgical time, and decreasing intraoperative and postoperative complications.

**ADVANCED COLLAGEN CROSS-LINKING**

The human cornea's optical transparency and mechanical strength are attributed to the well-organized matrix architecture composed of approximately 200 parallel sheets of narrow-diameter collagen fibrils arranged orthogonal to neighboring fibril sheets. Corneal fibrils are primarily composed of Type I collagen co-assembled with Type V collagen. However, there are other minor elements such as fibril-associated collagens with interrupted triple helices (FACIT) and small leucine-rich proteoglycans (SLRP) that modify the structure and function of collagen fibrils and contribute to corneal integrity. FACITs include Types XII and XIV collagen, and primary SLRPs include decorin, lumican, keratocan, and mimican. SLRP molecules such as decorin are critical for maintaining corneal transparency and corneal strength.

A weakened, ectatic cornea may be strengthened by linking its collagen fibers. These can be accomplished by treatment with chemicals, heat, ultraviolet irradiation, and interaction with naturally occurring molecules. Many reagents are capable of cross-linking collagen such as glutaraldehyde, glyceraldehyde, dianhydrides, and bis-isocyanates. In addition, collagens and collagen-based materials have been cross-linked using carbodiimides and poly(glycidyl) ether. However, these chemicals have not been specifically used to cross-link intact body tissues and are quite toxic. Formulations containing serum albumin and glutaraldehyde are approved to seal vascular anastomosis (BioGlue [CryoLife Inc, Kennesaw, GA]). However, their intended use is not to cross-link treated tissues.

It was noted in the early 1960s that exposure to ultraviolet light altered collagen. Specifically, 254-nm UV increased tensile strength by the induction of collagen cross-links. However, the effects of ultraviolet irradiation are extremely variable, ranging from polymerization to depolymerization depending on many conditions including wavelength, intensity, exposure time, and distance from the light source to the collagen composition. Riboflavin is well known as a photoinitiator for photopolymerization of acrylic monomers using riboflavin as the initiating redox system in the presence of oxygen. Delzenne et al studied the kinetics of polymerization of acrylic monomers using riboflavin and suggested that collagen aggregation was accompanied by loss of tyrosine and formation of dityrosine. More recently, Seiler and Spoerl successfully utilized riboflavin as a photoinitiator for ultraviolet light (UVA) treatment of the cornea to cross-link corneal tissue. This technique is currently being evaluated in a worldwide clinical study to evaluate the safety and efficacy of riboflavin/UVA light corneal cross-linking in patients with progressive keratoconus or corneal ectasia after previous refractive surgery.

**Novel Methods to Strengthen Corneal Structure**

Porcine eyes were treated with acetic anhydride and then were exposed to either 1, 3, and 4 10-second bursts of UV light at an intensity of 1000 mW at a band pattern of 310 to 400 nm; there was a 10-second period of non-exposure between UV bursts. The eyes were flushed with neutral pH phosphate buffer and mounted in a stress/strain analyzer. A single exposure increased the strength by 400%.

A similar study was performed on feline eyes using the Reichert Ocular Response Analyzer (ORA) to measure corneal hysteresis. Corneas were pretreated with a photoinitiator, 0.05 M sodium persulfate, and then exposed to 33.7 mW/cm² UV irradiation for 2 or 4 seconds. Results are shown in Table 18-1. There was a slight decrease in CH for the control eye at 60 days, a dramatic increase in CH for the 2-second UV-treated eye immediately after treatment, and a 11unit increase after 60 days. The 4-second UV-treated eye demonstrated a dramatic decrease in CH immediately following exposure. However, after 60 days, the CH returned to near baseline levels.

**Stabilization of Cornea by Application of Human Recombinant Decorin**

Decorin is a member of a family of small leucine-rich repeat proteoglycans (SLRPs). Decorin is an approximately 100 kDa proteoglycan consisting of a 40 kDa core protein and 1 chondroitin sulfate or dermatan sulfate glycosaminoglycan chain. Decorin interacts with collagen Type I and II, fibronectin, thrombospondin, and TGFβ.

Decorin is a horse-shoe shaped proteoglycan that binds to collagen fibrils in human cornea forming a bidentate ligand attached to 2 neighboring collagen molecules in the fibril or in adjacent fibrils, helping to stabilize fibrils and orient fibrillogenesis. Decorin appears to be a ubiquitous component of extracellular matrices linking collagen fibrils at specific binding sites. Corneal transparency is dependent on the size and arrangement of collagen fibrils in the corneal stroma. Decorin binding is critical in limiting collagen fibril growth and in controlling the arrangement of collagen fibrils to produce a transparency. Studies of effects of decorin on in vitro collagen fibrillogenesis show...
inhibition of collagen fibril growth and increase in collagen fibril diameter.\textsuperscript{29}

Stabilization occurs due to ionic binding of these molecules between adjacent collagen fibrils, forming a cross-link (or bridge) between such fibers. However, the penetration of these extracellular matrix molecules is limited due to the fact that the intrinsic conjunctival epithelial tissue layer forms tight junctions with high resistance to ocular delivery of hydrophilic molecules greater than 500 daltons. In addition, binding sites for the exogenous stabilization molecules on collagen fibers are limited because the sites are inherently occupied by natural extracellular matrix molecules.

Thus, in order to enhance delivery of stabilization agents and thereby maximize stromal stabilization, methods have been developed to 1) open the epithelium to allow intrastromal penetration of the stabilization agents and 2) dissociate inherent binding of extracellular molecules between adjacent collagen fibers in stromal tissue.

A chemical enhancer was developed to allow penetration of decorin. Confocal micrographs shown in Figure 18-12 demonstrate the ability of the penetration enhancer agent to disrupt epithelial cell junctures so that human recombinant decorin (MW approximately 40,000 daltons) can diffuse into the cornea following direct application to the central cornea. As shown, decorin penetration into the control cornea was limited to the epithelium when it was applied directly to the central cornea, and the epithelial cell junctures were not disrupted. Conversely, decorin penetrated the corneal stroma of the cornea pretreated with a penetration enhancer agent. Thus, penetration enhancer agent successfully disrupted the epithelial cell junctures, permitting diffusion of the 40,000 dalton decorin molecules.

### Transmission Electron Microscopy to Observe Decorin Binding to Collagen Fibers in a Feline Model

One eye from each group was treated with decorin. Decorin penetration into the corneal stroma was determined utilizing transmission electron microscopy. Corneas treated with decorin were reacted with Quinolinic Blue Stain (Cupromeronic blue) in buffered formalin. This reagent stains small proteoglycan structures such as decorin.

As shown in Figure 18-13, increased bridges were noted between collagen fibrils in the decorin-treated eyes.

Decorin also increased corneal hysteresis in the feline model as shown in Table 18-2. As shown, treatments with the chemical penetration enhancer reduced corneal hysteresis (CH), indicating “softening” of corneal structure due to dissociation of molecular links between collagen fibers. Subsequent application of decorin increased CH values to levels greater than initial values, indicating “strengthening” of corneal structure.

A second study was conducted to examine the effects of decorin treatment on stabilizing “weakened” cornea. Four

### Table 18-1

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<th>CONDITIONS</th>
<th>CH VALUES</th>
<th>Unit Change at 2 Months</th>
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<tr>
<td>Control-no UV</td>
<td>Baseline: 4.5</td>
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<tr>
<td></td>
<td>Immediately Post-Treat: 6.34</td>
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<td>60-Days Post-Treat: 7.53</td>
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**Figure 18-12.** Confocal micrographs demonstrate the ability of the penetration enhancer agent to disrupt epithelial cell junctures so that human recombinant decorin (MW approximately 40,000 daltons) can diffuse into the cornea following direct application to the central cornea.
subject animals were included in this study. One eye of each animal was treated with the contralateral eye serving as the control. Eyes were evaluated for corneal hysteresis using the Reichert Ocular Response Analyzer before treatment and then after treatment with proparacaine HCL, ocular penetration enhancer, and decorin. As shown in Table 18-3, treatments with proprietary ocular enhancer solution reduced CH values, indicating corneal softening. Subsequent application of human recombinant decorin increased CH values, indicating stabilization of corneal structure.

A third study was conducted to examine the effects of decorin on strengthening or stabilizing corneal structural integrity using the Reichert Ocular Response Analyzer. Two subject animals were included in this study. One eye of each animal was treated while the contralateral eye served as the control. Control and treated eyes were evaluated for corneal hysteresis using the Reichert Ocular Response Analyzer at baseline and then after treatment with Proparacaine HCL, proprietary ocular enhancer solution, and decorin.
Corneal hysteresis (CH) results are shown in Table 18-4. As in previous studies, the corneal hysteresis values increased following treatment with human recombinant decorin solution.

These studies suggest that topical decorin may be valuable in the treatment of keratoconus and post-LASIK ectasia. It may also be possible to reshape the cornea using decorin and orthokeratology lenses to correct refractive errors. Finally, it is conceivable that decorin or similar molecules can be used to delay presbyopia by altering the cornea or scleral architecture.

REFERENCES


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